# New insights into acquired temozolomide resistance in glioblastoma?

Happold, C ; Weller, M

DOI: https://doi.org/10.1093/brain/awv301

MSA. Neuropathol Appl Neurobiol 2007; 33: 615–20.
Wenning GK, Colosimo C, Geser F, Poewe W. Multiple system atrophy. Lancet Neurol 2004; 3: 93–103.
Wenning GK, Geser F, Krismer F, Seppi K, Duerr S, Boesch S, et al. European Multiple System Atrophy Study Group. The natural history of multiple system atrophy: a prospective European cohort study. Lancet Neurol 2013; 12: 264–74.

# New insights into acquired temozolomide resistance in glioblastoma?

**This scientific commentary refers to 'c-Myc–miR-29c–REV3L signalling pathway drives the acquisition of temozolomide resistance in glioblastoma' by Luo *et al.* (doi:10.1093/brain/awv287)**

The emergence of tumour cell resistance to chemotherapy represents a major challenge for the development of durable therapeutic strategies across most solid cancers including glioblastoma, the most malignant primary brain tumour in adults. The standard of care for glioblastoma is maximum surgery as safely feasible followed by radiotherapy, with concomitant and maintenance chemotherapy with the alkylating agent temozolomide (TMZ/RT→TMZ). This results in a median overall survival in the range of 12 months on a population level (Weller *et al.*, 2014). Radiotherapy alone doubled median survival in early studies, but the best radiological response is commonly stable disease, progression is inevitable, and the efficacy of radiotherapy may be partly related to anti-angiogenic rather than intrinsic tumour cell cytotoxic effects. Mechanisms underlying resistance to radiotherapy remain poorly understood, but extensive hypoxia may be an important factor.

TMZ was approved for the treatment of newly diagnosed glioblastoma because of a moderate prolongation of median survival (Stupp *et al.*, 2005). Subgroup analyses revealed that the benefit from TMZ was largely restricted to patients with glioblastomas that exhibit a particular epigenetic alteration, promoter methylation of the $O^6$-methylguanine DNA methyltransferase (*MGMT*) gene (Hegi *et al.*, 2005). However, even these patients eventually all progress and succumb to their disease, in the absence of changes in *MGMT* promoter methylation (Felsberg *et al.*, 2011), indicating that novel pathways must be activated to escape from alkylating agent chemotherapy.

Considerable efforts have been made to understand and overcome glioma cell resistance to chemotherapy, focusing on several proteins and pathways involved in cell survival and resistance, including the mismatch repair (MMR) pathway. In this issue of *Brain*, Luo and co-workers report that a c-Myc driven downregulation of microRNA (miR)-29c promotes a TMZ-resistant phenotype of glioma cells that is mediated by increased *REV3L* expression and consequent enhanced DNA repair capacity (Luo *et al.*, 2015).

MicroRNAs have been studied extensively over the last decade for their role in diverse biological processes, especially in cell proliferation and cell death. These small non-coding RNA molecules can affect post-transcriptional control of gene expression and may thereby help to regulate several aspects of tumorigenesis, including invasiveness, DNA repair, and acquired resistance to genotoxic cancer therapy. MicroRNAs can function as either tumour suppressors or promoters, in a context-dependent manner. While downregulation of miR-29c has been associated with tumorigenesis and invasiveness in a variety of cancers, including gliomas (Fan *et al.*, 2013; Wang *et al.*, 2013), no link to TMZ resistance has been established so far.

Here, Luo and co-workers used quantitative PCR and *in situ* hybridization to confirm a downregulation of miR-29c—which was selected from an array-based analysis of matched primary and recurrent human gliomas considered TMZ-resistant—in recurrent tumour samples and cell lines selected for TMZ resistance (U251/TMZR, U87/TMZR). Restoration of miR-29c expression restored TMZ sensitivity. By contrast, inhibition of miR-29c in A172 cells, which express high levels of miR-29c comparable to those of normal human astrocytes, conferred TMZ resistance. These effects were reproduced in an *in vivo* model of non-obese diabetic (NOD)-severe combined immunodeficiency (SCID) mice injected subcutaneously with resistant U251/TMZR cells. Prolonged survival was noted in animals treated with intraperitoneal TMZ at 20 mg/kg and simultaneous intratumoural injection of miR-29c (1 nmol), applied as cholesterol-conjugated 2'-O-methylmodified miR-29c for better pharmacokinetic properties.

To assess potential target genes that could be mediating the effect of miR-29c, Luo *et al.* used a somewhat artificial or at least unconventional approach: they generated a list of candidate genes that were (i) deregulated in TMZ-resistant cell lines that had been engineered to overexpress miR-29c; and (ii) also predicted targets of miR-29c according to public databases. They identified REV3L, a DNA repair polymerase previously

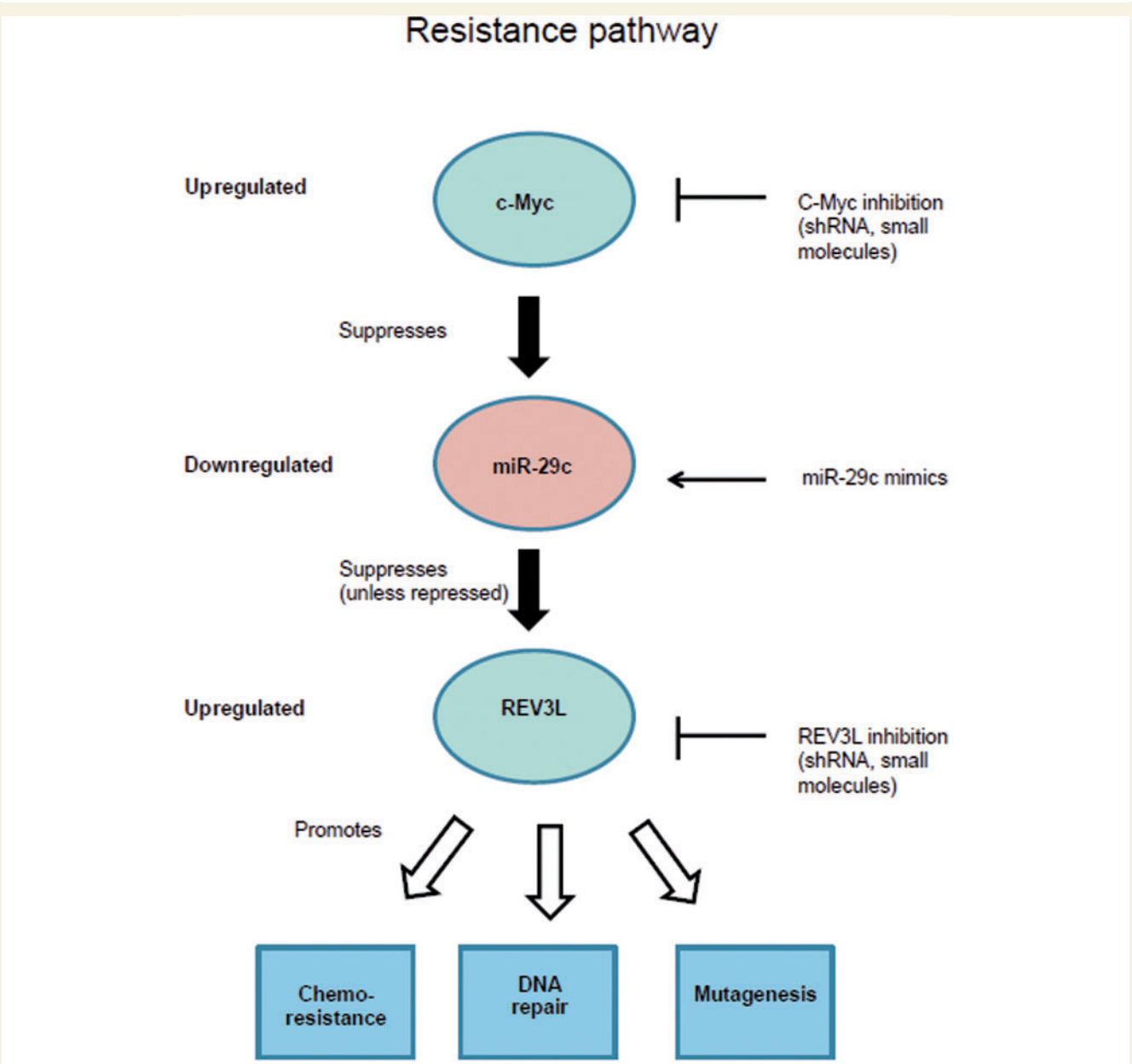


**Figure 1 Proposed mechanism of TMZ resistance in glioblastoma.** Upregulation of the miR-29c suppressor c-Myc leads to increased expression of REV3L, resulting in enhanced DNA repair and chemoresistance. Potential approaches to counteract this pathway are indicated on the right.

linked to TMZ resistance (Roos *et al.*, 2009), as less strongly expressed in the resistant cells with ectopic miR-29c expression; accordingly, inhibition of miR-29c by commercially available antisense miR-29c molecules in A172 cells led to an increase in REV3L levels. A luciferase reporter assay confirmed direct targeting of the 3'UTR on the *REV3L* mRNA by miR-29c.

In an intracranial mouse model consisting of animals injected with primary GBM-2 or GBM-4 glioblastoma cells, prior transfection of the tumour cells with miR-29c-mimics reduced REV3L levels determined by immunohistochemistry, reduced tumour burden and increased survival when the mice were treated with TMZ. Moreover, when short hairpin (sh)REV3L was used to block *REV3L* expression in resistant GBM-2 and GBM-4 cells expressing anti-miR-29c, a sensitizing effect to TMZ was noted. This was shown *in vitro* by decreased colony formation ability and increased apoptosis, and *in vivo* by reduced tumour volume. The most likely mechanism by which *REV3L* overexpression produces TMZ resistance is through sustained DNA repair, since U87 scramble-transduced cells showed more rapid DNA repair than U87 cells with miR-29c overexpression or *REV3L* gene silencing. These cells exhibited increased levels of $\gamma$-H2AX foci, a reaction to DNA double-strand breaks, and

chromosomal breakage in response to TMZ.

Lastly, the authors identify c-Myc, a transcription factor involved in cell cycle progression and apoptosis that is often constitutively expressed in cancer cells, as an important positive upstream regulator of the newly described miR-29c/REV3L axis, acting as an miR-29c suppressor and thus indirect REV3L inducer. Taken together, therefore, Luo *et al*. propose a signalling axis whereby TMZ resistance mediated by improved DNA repair is achieved through c-Myc-induced suppression of miR-29c with consecutive *REV3L* induction (Fig. 1). The clinical relevance of these findings was underlined by a Kaplan-Meier survival analysis of 138 glioblastoma patients treated according to standard of care (TMZ/RT→TMZ). Patients with higher miR-29c levels reportedly had better survival rates. Of note, the tissue specimens with high miR-29c expression had lower levels of c-Myc and REV3L, as predicted from the *in vitro* findings.

This study has some limitations, including the use of a subcutaneous rather than intracranial tumour model in some experiments, the artificial approach to identifying REV3L as a target of miR-29c, and the lack of cross-resistance to explore whether this pathway is specific to TMZ or is relevant for other alkylators such as CCNU, too, or for drugs with different modes of action, or even for radiotherapy.

Importantly, the regulation of TMZ resistance reported here was observed in primary glioblastoma cells with or without *MGMT* expression. Accordingly, no regulation of MGMT levels was observed when cells were exposed to miR-29c mimics or inhibitors. Moreover, no role was identified for the MMR pathway in this resistance either. Thus, the interaction of miR-29c and REV3L could represent an interesting therapeutic target for all glioblastomas, with or without *MGMT* promoter methylation, provided that this network can be selectively targeted in neoplastic cells.

Future studies should explore which factors regulate c-Myc expression upstream of this resistance pathway. Knowledge of this upstream mechanism and factors modulating the downstream cascade would allow for potential therapeutic interventions, focusing for example on clinically applicable microRNA mimics or REV3L inhibitors.

*Caroline Happold and Michael Weller*
*Department of Neurology, University Hospital and University of Zurich, Zurich, Switzerland*

Correspondence to: Michael Weller
E-mail: michael.weller@usz.ch

doi:10.1093/brain/awv301

## References

Fan YC, Mei PJ, Chen C, Miao FA, Zhang H, Li ZL. MiR-29c inhibits glioma cell proliferation, migration, invasion and angiogenesis. J Neurooncol 2013; 115: 179–88.

Felsberg J, Thon N, Eigenbrod S, Hentschel B, Sabel MC, Westphal M, et al. Promoter methylation and expression of MGMT and the DNA mismatch repair genes MLH1, MSH2, MSH6 and PMS2 in paired primary and recurrent glioblastomas. Int J Cancer 2011; 129: 659–70.

Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005; 352: 997–1003.

Luo H, Chen Z, Wang S, Zhang R, Qiu W, Zhao L, et al. c-Myc-miR-29c-REV3L signaling pathway drives the acquisition of temozolomide resistance in glioblastoma. Brain 2015; 138: 3654–3672.

Roos WP, Tsaalbi-Shtylik A, Tsaryk R, Guvercin F, de Wind N, Kaina B. The translesion polymerase Rev3L in the tolerance of alkylating anticancer drugs. Mol Pharmacol 2009; 76: 927–34.

Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005; 352: 987–96.

Wang Y, Li Y, Sun J, Wang Q, Sun C, Yan Y, et al. Tumor-suppressive effects of miR-29c on gliomas. Neuroreport 2013; 24: 637–45.

Weller M, van den Bent M, Hopkins K, Tonn JC, Stupp R, Falini A, et al. EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. Lancet Oncol 2014; 15: e395–403.

# Lipidated APOE has effects on cognitive function that are independent of amyloid-β pathology

**This scientific commentary refers to 'Opposing effects of *Apoe/Apoa1* double deletion on amyloid-β pathology and cognitive performance in APP mice', by Fitz *et al.* (doi:10.1093/brain/awv293).**

Since the discovery of the apolipoprotein E ε4 allele (*APOE4*) as the strongest genetic risk factor for Alzheimer's disease (reviewed by Verghese *et al*., 2011), there has been a dramatic increase in research on the intersection of lipid biology and Alzheimer pathology. APOE is the major cholesterol and lipid carrier in the brain, while apolipoprotein A1 (APOA1) is the major lipid carrier in the periphery. Efflux of cholesterol and